FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
DEX-0150	09/762021

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/16357	**20 July 1999**	**4 August 1998**

TITLE OF INVENTION

A Novel Method of Diagnosing, Monitoring, Staging, Imaging and Treating Colon Cancer

APPLICANT(S) FOR DO/EO/US

SUN, Yongming et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1)
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau)
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). - Unexecuted
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

1) Courtesy copy of International Application
2) Executed Verified Statement Claiming Small Entity Status
3) Return Post Card

"Express Mail" Label No. **EL722986068US**
Date of Deposit **February 1, 2001**

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231.

By Deborah Ehret
Typed Name: Deborah Ehret

#7

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.:	**DEX-0150**
Inventors:	**Sun et al.**
Serial No.:	**09/762,021**
Filing Date:	**February 1, 2001**
Examiner:	**Not yet assigned**
Group Art Unit:	**Not yet assigned**
Title:	**A Novel Method of Diagnosing, Monitoring, Staging, Imaging and Treating Colon Cancer**

BOX SEQUENCE
Assistant Commissioner for Patents
Washington, DC 20231

Sir:

AMENDMENT

In reply to the Notication to Comply with Requirements for Patent Applications containing Nucleotide Sequence and/or Amino Acid Sequence Disclosure dated **August 2, 2001**, a response to which is due **September 2, 2001**, it is requested that the Sequence Listing of the instant specification be deleted and replaced with the amended Sequence Listing provided herewith. The replacement Sequence Listing has been amended to conform with the current Sequence Listing Rules. However, data presented in the Sequence

Listing is identical to that originally filed. Thus, no new matter has been added by this amendment.

Respectfully submitted,

Kathleen A. Tyrrell
Registration No. 38,350

Date: **September 4,2001**

LICATA & TYRRELL P.C.
66 E. Main Street
Marlton, New Jersey 08053

(856) 810-1515

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.:	DEX-0150
Inventors:	Sun et al.
Serial No.:	09/762,021
Filing Date:	February 1, 2001
Examiner:	Not yet assigned
Group Art Unit:	Not yet assigned
Title:	A Novel Method of Diagnosing, Monitoring, Staging, Imaging and Treating Colon Cancer

"Express Mail" Label No. **EL 722985500 US**
Date of Deposit - **September 4, 2001**

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By _____
Typed Name: **Kathleen A. Tyrrell**

BOX SEQUENCE
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE WITH 37 CFR §§ 1.821 THROUGH 1.825

( ) I hereby state, in accordance with the requirements of **37 C.F.R. §1.821(f)**, that the contents of the paper and computer readable copies of the Sequence Listing, submitted in accordance with **37 CFR §1.821(c)** and **(e)**, respectively are the same.

( ) I hereby state that the submission filed in accordance with **37 CFR §1.821(g)** does not include new matter.

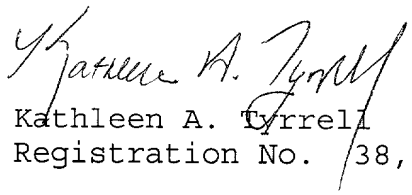
( ) I hereby state that the submission filed in accordance with **37 CFR §1.821(h)** does not include new matter or go beyond the disclosure in the international application as filed.

(XX) I hereby state that the amendments, made in accordance with **37 CFR §1.825(a)**, included in the substitute sheet(s) of the Sequence Listing were made to conform with the current Sequence Listing rules. I hereby state that the substitute sheet(s) of the Sequence Listing does not include new matter.

(XX) I hereby state that the substitute copy of the computer readable form, submitted in accordance with **37 CFR §1.825(b)**, is the same as the amended Sequence Listing.

(XX) I hereby state that the substitute copy of the computer readable form, submitted in accordance with **37 CFR §1.825(d)**, contains identical data to that originally filed.

Respectfully submitted,



Kathleen A. Tyrrell
Registration No. 38,350

Date: **September 4, 2001**

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UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents, Box PCT
United States Patent and Trademark Office
Washington, D C 20231
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U.S. APPLICATION NO		FIRST NAMED APPLICANT	ATTY DOCKET NO
09/762021		SUN Y	DEX-0150

INTERNATIONAL APPLICATION NO
PCT/US99/16357

35 U.S.C. 371 FILING DATE	PRIORITY DATE
20 JUL 99	04 AUG 98

LICATA & TYRRELL
66 E MAIN STREET
MARLTON, NJ 08054

Docket System ✓
Status Report ✓
Docket Book ✓

DATE MAILED 02 AUG 2001

Notice to Comply 9-2-01 2-2-02

NOTIFICATION TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

Applicant has submitted papers under 35 U.S.C. 371 to enter the national stage in the United States of America. The items indicated below, however, are missing. The period within which to correct the deficiency noted below and avoid abandonment is set forth in the accompanying Notification.

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 CFR 1.821-1.825 for the following reason(s):

- [x] The application fails to comply with the requirements of 37 CFR 1.821-1.825.
- [ ] This application does not contain, a "Sequence Listing" as a separate part of the disclosure on paper copy or compact disc, as required by 37 CFR 1.821(c).
- [ ] A copy of the "Sequence Listing" in computer readable format has not been submitted as required by 37 CFR 1.821(e).
- [x] A copy of the "Sequence Listing" in computer readable form has been submitted. The content of the computer readable form, however, does not comply with the requirements of 37 CFR 1.822 and/or 1.832, as indicated on the attached marked-up copy of the "Raw Sequence Listing."
- [ ] The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A substitute computer readable form must be submitted as required by 37 CFR 1.825(d).
- [ ] The paper copy or compact disc of the "Sequence Listing" is not the same as the computer readable form of the "Sequence Listing" as required by 37 CFR 1.821(e)
- [ ] Other: _____

_____

APPLICANT MUST PROVIDE:

- [x] An initial or substitute computer readable form (CRF) of the "Sequence Listing."
- [ ] An initial or substitute paper copy or compact disc of the "Sequence Listing," as well as an amendment directing its entry into the specification.
- [x] A statement that the contents of the paper or compact disc and the computer readable form are the same and, where applicable, include no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b) or 1.825(d).

FOR QUESTIONS REGARDING COMPLIANCE WITH THESE REQUIREMENTS, PLEASE CALL:

(703) 308-4216, for Rules interpretation,
(703) 308-4212, for CRF submission help,
(703) 287-0200, for PatentIn software help.

Barbara A. Campbell
Telephone 703-305-3631

FORM PCT/DO/EO/920 (March 2001)

SEQUENCE LISTING

<110> Sun, Yongming
Macina, Roberto A
Recipon, Herve
DIADEXUS LLC

<120> A NOVEL METHOD OF DIAGNOSING, MONITORING, STAGING, IMAGING AND TREATING COLON CANCER

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A NOVEL METHOD OF DIAGNOSING, MONITORING, STAGING, IMAGING AND TREATING COLON CANCER

FIELD OF THE INVENTION

This invention relates, in part, to newly developed assays for detecting, diagnosing, monitoring, staging, prognosticating, imaging and treating cancers, particularly colon cancer.

BACKGROUND OF THE INVENTION

Cancer of the colon is a highly treatable and often curable disease when localized to the bowel. It is one of the most frequently diagnosed malignancy in the United States as well as the second most common cause of cancer death. Surgery is the primary treatment and results in cure in approximately 50% of patients. However, recurrence following surgery is a major problem and often is the ultimate cause of death.

The prognosis of colon cancer is clearly related to the degree of penetration of the tumor through the bowel wall and the presence or absence of nodal involvement. These two characteristics form the basis for all staging systems developed for this disease. Treatment decisions are usually made in reference to the older Duke's or the Modified Astler-Coller (MAC) classification scheme for staging.

Bowel obstruction and bowel perforation are indicators of poor prognosis in patients with colon cancer. Elevated pretreatment serum levels of carcinoembryonic antigen (CEA) and of carbohydrate antigen 19-9 (CA 19-9) also have a negative prognostic significance.

Age greater than 70 years at presentation is not a contraindication to standard therapies. Acceptable morbidity and mortality, as well as long-term survival, are achieved in this patient population.

Because of the frequency of the disease (approximately 160,000 new cases of colon and rectal cancer per year), the identification of high-risk groups, the demonstrated slow growth of primary lesions, the better survival of early-stage lesions, and the relative simplicity and accuracy of screening tests, screening for colon cancer should be a part of routine care for all adults starting at age 50, especially those with first-degree relatives with colorectal cancer.

Procedures used for detecting, diagnosing, monitoring, staging, and prognosticating colon cancer are of critical importance to the outcome of the patient. For example, patients diagnosed with early colon cancer generally have a much greater five-year survival rate as compared to the survival rate for patients diagnosed with distant metastasized colon cancer. New diagnostic methods which are more sensitive and specific for detecting early colon cancer are clearly needed.

Colon cancer patients are closely monitored following initial therapy and during adjuvant therapy to determine response to therapy and to detect persistent or recurrent disease of metastasis. There is clearly a need for a colon cancer marker which is more sensitive and specific in detecting colon cancer, its recurrence, and progression.

Another important step in managing colon cancer is to determine the stage of the patient's disease. Stage determination has potential prognostic value and provides criteria for designing optimal therapy. Generally, pathological staging of colon cancer is preferable over clinical staging because the former gives a more accurate prognosis. However, clinical staging would be preferred were it at least as accurate as pathological staging because it does not depend on an invasive procedure to obtain tissue for pathological evaluation. Staging of colon cancer would be improved by detecting new markers in cells, tissues, or bodily

fluids which could differentiate between different stages of invasion.

In the present invention methods are provided for detecting, diagnosing, monitoring, staging, prognosticating, *in vivo* imaging and treating colon cancer via three (3) Colon Specific Genes (CSGs). The 3 CSGs refer, among other things, to native proteins expressed by the genes comprising the polynucleotide sequences of any of SEQ ID NO: 1, 2, or 3. In the alternative, what is meant by the 3 CSGs as used herein, means the native mRNAs encoded by the genes comprising any of the polynucleotide sequences of SEQ ID NO: 1, 2, or 3 or it can refer to the actual genes comprising any of the polynucleotide sequences of SEQ ID NO: 1, 2, or 3.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following description and from reading the other parts of the present disclosure.

SUMMARY OF THE INVENTION

Toward these ends, and others, it is an object of the present invention to provide a method for diagnosing the presence of colon cancer by analyzing for changes in levels of CSG in cells, tissues or bodily fluids compared with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control, wherein an increase in levels of CSG in the patient versus normal human control is associated with colon cancer.

Further provided is a method of diagnosing metastatic colon cancer in a patient having such cancer which is not

known to have metastasized by identifying a human patient suspected of having colon cancer that has metastasized; analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissues, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

Also provided by the invention is a method of staging colon cancer in a human which has such cancer by identifying a human patient having such cancer; analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing CSG levels in such cells, tissues, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CSG is associated with a cancer which is regressing or in remission.

Further provided is a method of monitoring colon cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

Further provided is a method of monitoring the change in stage of colon cancer in a human having such cancer by looking at levels of CSG in a human having such cancer. The method comprises identifying a human patient having such

cancer; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CSG is associated with a cancer which is regressing or in remission.

Further provided are antibodies against the CSGs or fragments of such antibodies which can be used to detect or image localization of the CSGs in a patient for the purpose of detecting or diagnosing a disease or condition. Such antibodies can be polyclonal or monoclonal, or prepared by molecular biology techniques. The term "antibody", as used herein and throughout the instant specification is also meant to include aptamers and single-stranded oligonucleotides such as those derived from an *in vitro* evolution protocol referred to as SELEX and well known to those skilled in the art. Antibodies can be labeled with a variety of detectable labels including, but not limited to, radioisotopes and paramagnetic metals. These antibodies or fragments thereof can also be used as therapeutic agents in the treatment of diseases characterized by expression of a CSG. In therapeutic applications, the antibody can be used without or with derivatization to a cytotoxic agent such as a radioisotope, enzyme, toxin, drug or a prodrug.

Other objects, features, advantages and aspects of the present invention will become apparent to those of skill in the art from the following description. It should be understood, however, that the following description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only. Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those

skilled in the art from reading the following description and from reading the other parts of the present disclosure.

DESCRIPTION OF THE INVENTION

The present invention relates to diagnostic assays and methods, both quantitative and qualitative for detecting, diagnosing, monitoring, staging, and prognosticating cancers by comparing levels of CSG with those of CSG in a normal human control. What is meant by levels of CSG as used herein, means levels of the native protein expressed by the genes comprising the polynucleotide sequence of any of SEQ ID NO: 1, 2, or 3. In the alternative, what is meant by levels of CSG as used herein, means levels of the native mRNA encoded by any of the genes comprising any of the polynucleotide sequences of SEQ ID NO: 1, 2, or 3 or levels of the gene comprising any of the polynucleotide sequence of SEQ ID NO: 1, 2, or 3. Such levels are preferably measured in at least one of, cells, tissues and/or bodily fluids, including determination of normal and abnormal levels. Thus, for instance, a diagnostic assay in accordance with the invention for diagnosing over-expression of any one of the CSG proteins compared to normal control bodily fluids, cells, or tissue samples may be used to diagnose the presence of cancers, including colon cancer. Any of the 3 CSGs may be measured alone in the methods of the invention, or all together or any combination of the three.

All the methods of the present invention may optionally include measuring the levels of other cancer markers as well as CSG. Other cancer markers, in addition to CSG, useful in the present invention will depend on the cancer being tested and are known to those of skill in the art.

Diagnostic Assays

The present invention provides methods for diagnosing the presence of colon cancer by analyzing for changes in levels of CSG in cells, tissues or bodily fluids compared with levels

of CSG in cells, tissues or bodily fluids of preferably the same type from a normal human control, wherein an increase in levels of CSG in the patient versus the normal human control is associated with the presence of colon cancer.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the patient being tested has cancer is one in which cells, tissues, or bodily fluid levels of the cancer marker, such as CSG, are at least two times higher, and most preferable are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal human control.

The present invention also provides a method of diagnosing metastatic colon cancer in a patient having colon cancer which has not yet metastasized for the onset of metastasis. In the method of the present invention, a human cancer patient suspected of having colon cancer which may have metastasized (but which was not previously known to have metastasized) is identified. This is accomplished by a variety of means known to those of skill in the art. For example, in the case of colon cancer, patients are typically diagnosed with colon cancer following traditional detection methods.

In the present invention, determining the presence of CSG level in cells, tissues, or bodily fluid, is particularly useful for discriminating between colon cancer which has not metastasized and colon cancer which has metastasized. Existing techniques have difficulty discriminating between colon cancer which has metastasized and colon cancer which has not metastasized and proper treatment selection is often dependent upon such knowledge.

In the present invention, the cancer marker levels measured in such cells, tissues, or bodily fluid is CSG, and are compared with levels of CSG in preferably the same cells, tissue, or bodily fluid type of a normal human control. That is, if the cancer marker being observed is just CSG in serum,

this level is preferably compared with the level of CSG in serum of a normal human patient. An increase in the CSG in the patient versus the normal human control is associated with colon cancer which has metastasized.

Without limiting the instant invention, typically, for a quantitative diagnostic assay a positive result indicating the cancer in the patient being tested or monitored has metastasized is one in which cells, tissues, or bodily fluid levels of the cancer marker, such as CSG, are at least two times higher, and most preferable are at least five times higher, than in preferably the same cells, tissues, or bodily fluid of a normal patient.

Normal human control as used herein includes a human patient without cancer and/or non cancerous samples from the patient; in the methods for diagnosing metastasis or monitoring for metastasis, normal human control preferably includes samples from a human patient that is determined by reliable methods to have colon cancer which has not metastasized such as earlier samples from the same patient prior to metastasis.

Staging

The invention also provides a method of staging colon cancer in a human patient.

The method comprises identifying a human patient having such cancer; analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG. Then, the method compares CSG levels in such cells, tissues, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which is progressing and a decrease in the levels of CSG is associated with a cancer which is regressing or in remission.

Monitoring

Further provided is a method of monitoring colon cancer in a human having such cancer for the onset of metastasis. The method comprises identifying a human patient having such cancer that is not known to have metastasized; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

Further provided by this inventions is a method of monitoring the change in stage of colon cancer in a human having such cancer. The method comprises identifying a human patient having such cancer; periodically analyzing a sample of cells, tissues, or bodily fluid from such patient for CSG; comparing the CSG levels in such cells, tissue, or bodily fluid with levels of CSG in preferably the same cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in CSG levels in the patient versus the normal human control is associated with a cancer which is progressing in stage and a decrease in the levels of CSG is associated with a cancer which is regressing in stage or in remission.

Monitoring such patient for onset of metastasis is periodic and preferably done on a quarterly basis. However, this may be more or less frequent depending on the cancer, the particular patient, and the stage of the cancer.

Assay Techniques

Assay techniques that can be used to determine levels of gene expression, such as CSG of the present invention, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays,

reverse transcriptase PCR (RT-PCR) assays, immunohistochemistry assays, *in situ* hybridization assays, competitive-binding assays, Western Blot analyses, ELISA assays and proteomic approaches. Among these, ELISAs are frequently preferred to diagnose a gene's expressed protein in biological fluids.

An ELISA assay initially comprises preparing an antibody, if not readily available from a commercial source, specific to CSG, preferably a monoclonal antibody. In addition a reporter antibody generally is prepared which binds specifically to CSG. The reporter antibody is attached to a detectable reagent such as radioactive, fluorescent or enzymatic reagent, for example horseradish peroxidase enzyme or alkaline phosphatase.

To carry out the ELISA, antibody specific to CSG is incubated on a solid support, e.g., a polystyrene dish, that binds the antibody. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the sample to be analyzed is incubated in the dish, during which time CSG binds to the specific antibody attached to the polystyrene dish. Unbound sample is washed out with buffer. A reporter antibody specifically directed to CSG and linked to horseradish peroxidase is placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to CSG. Unattached reporter antibody is then washed out. Reagents for peroxidase activity, including a colorimetric substrate are then added to the dish. Immobilized peroxidase, linked to CSG antibodies, produces a colored reaction product. The amount of color developed in a given time period is proportional to the amount of CSG protein present in the sample. Quantitative results typically are obtained by reference to a standard curve.

A competition assay may be employed wherein antibodies specific to CSG attached to a solid support and labeled CSG

and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of CSG in the sample.

Nucleic acid methods may be used to detect CSG mRNA as a marker for colon cancer. Polymerase chain reaction (PCR) and other nucleic acid methods, such as ligase chain reaction (LCR) and nucleic acid sequence based amplification (NASABA), can be used to detect malignant cells for diagnosis and monitoring of various malignancies. For example, reverse-transcriptase PCR (RT-PCR) is a powerful technique which can be used to detect the presence of a specific mRNA population in a complex mixture of thousands of other mRNA species. In RT-PCR, an mRNA species is first reverse transcribed to complementary DNA (cDNA) with use of the enzyme reverse transcriptase; the cDNA is then amplified as in a standard PCR reaction. RT-PCR can thus reveal by amplification the presence of a single species of mRNA. Accordingly, if the mRNA is highly specific for the cell that produces it, RT-PCR can be used to identify the presence of a specific type of cell.

Hybridization to clones or oligonucleotides arrayed on a solid support (i.e., gridding) can be used to both detect the expression of and quantitate the level of expression of that gene. In this approach, a cDNA encoding the CSG gene is fixed to a substrate. The substrate may be of any suitable type including but not limited to glass, nitrocellulose, nylon or plastic. At least a portion of the DNA encoding the CSG gene is attached to the substrate and then incubated with the analyte, which may be RNA or a complementary DNA (cDNA) copy of the RNA, isolated from the tissue of interest. Hybridization between the substrate bound DNA and the analyte can be detected and quantitated by several means including but not limited to radioactive labeling or fluorescence labeling of the analyte or a secondary molecule designed to detect the hybrid. Quantitation of the level of gene expression can be

done by comparison of the intensity of the signal from the analyte compared with that determined from known standards. The standards can be obtained by *in vitro* transcription of the target gene, quantitating the yield, and then using that material to generate a standard curve.

Of the proteomic approaches, 2D electrophoresis is a technique well known to those in the art. Isolation of individual proteins from a sample such as serum is accomplished using sequential separation of proteins by different characteristics usually on polyacrylamide gels. First, proteins are separated by size using an electric current. The current acts uniformly on all proteins, so smaller proteins move farther on the gel than larger proteins. The second dimension applies a current perpendicular to the first and separates proteins not on the basis of size but on the specific electric charge carried by each protein. Since no two proteins with different sequences are identical on the basis of both size and charge, the result of a 2D separation is a square gel in which each protein occupies a unique spot. Analysis of the spots with chemical or antibody probes, or subsequent protein microsequencing can reveal the relative abundance of a given protein and the identity of the proteins in the sample.

The above tests can be carried out on samples derived from a variety of patients' cells, bodily fluids and/or tissue extracts (homogenates or solubilized tissue) such as from tissue biopsy and autopsy material. Bodily fluids useful in the present invention include blood, urine, saliva, or any other bodily secretion or derivative thereof. Blood can include whole blood, plasma, serum, or any derivative of blood.

In Vivo Antibody Use

Antibodies against CSG can also be used *in vivo* in patients with diseases of the colon. Specifically, antibodies

against an CSG can be injected into a patient suspected of having a disease of the colon for diagnostic and/or therapeutic purposes. The use of antibodies for *in vivo* diagnosis is well known in the art. For example, antibody-chelators labeled with Indium-111 have been described for use in the radioimmunoscintographic imaging of carcinoembryonic antigen expressing tumors (Sumerdon et al. Nucl. Med. Biol. 1990 17:247-254). In particular, these antibody-chelators have been used in detecting tumors in patients suspected of having recurrent colorectal cancer (Griffin et al. J. Clin. Onc. 1991 9:631-640). Antibodies with paramagnetic ions as labels for use in magnetic resonance imaging have also been described (Lauffer, R.B. Magnetic Resonance in Medicine 1991 22:339-342). Antibodies directed against CSGs can be used in a similar manner. Labeled antibodies against an CSG can be injected into patients suspected of having a disease of the colon such as colon cancer for the purpose of diagnosing or staging of the disease status of the patient. The label used will be selected in accordance with the imaging modality to be used. For example, radioactive labels such as Indium-111, Technetium-99m or Iodine-131 can be used for planar scans or single photon emission computed tomography (SPECT). Positron emitting labels such as Fluorine-19 can be used in positron emission tomography. Paramagnetic ions such as Gadlinium (III) or Manganese (II) can used in magnetic resonance imaging (MRI). Localization of the label within the colon or external to the colon permits determination of the spread of the disease. The amount of label within the colon also allows determination of the presence or absence of cancer in the colon.

For patients diagnosed with colon cancer, injection of an antibody against a CSG can also have a therapeutic benefit. The antibody may exert its therapeutic effect alone. Alternatively, the antibody is conjugated to a cytotoxic agent such as a drug, toxin or radionuclide to enhance its

therapeutic effect. Drug monoclonal antibodies have been described in the art for example by Garnett and Baldwin, *Cancer Research* 1986 46:2407-2412. The use of toxins conjugated to monoclonal antibodies for the therapy of various cancers has also been described by Pastan et al. *Cell* 1986 47:641-648). Yttrium-90 labeled monoclonal antibodies have been described for maximization of dose delivered to the tumor while limiting toxicity to normal tissues (Goodwin and Meares Cancer Supplement 1997 80:2675-2680). Other cytotoxic radionuclides including, but not limited to Copper-67, Iodine-131 and Rhenium-186 can also be used for labeling of antibodies against CSGs.

Antibodies which can be used in these *in vivo* methods include both polyclonal and monoclonal antibodies and antibodies prepared via molecular biology techniques. Antibody fragments and aptamers and single-stranded oligonucleotides such as those derived from an *in vitro* evolution protocol referred to as SELEX and well known to those skilled in the art can also be used.

EXAMPLES

The present invention is further described by the following example. The example is provided solely to illustrate the invention by reference to specific embodiments. These exemplifications, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

Example 1

Identification of CSGs were carried out by a systematic analysis of data in the LIFESEQ database available from Incyte Pharmaceuticals, Palo Alto, CA, using the data mining Cancer Leads Automatic Search Package (CLASP) developed by diaDexus LLC, Santa Clara, CA.

The CLASP performs the following steps:

Selection of highly expressed organ specific genes based on the abundance level of the corresponding EST in the targeted organ versus all the other organs.

Analysis of the expression level of each highly expressed organ specific genes in normal, tumor tissue, disease tissue and tissue libraries associated with tumor or disease.

Selection of the candidates demonstrating component ESTs were exclusively or more frequently found in tumor libraries.

CLASP allows the identification of highly expressed organ and cancer specific genes useful in the diagnosis of colon cancer.

Table 1: CSGs Sequences

SEQ ID NO:	LS Clone ID	LSA Gene ID
1	1517021	236347
2	776410	202109
3	611082	202298

The following Example was carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. Routine molecular biology techniques of the following example can be carried out as described in standard laboratory manuals, such as Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

Example 2: Relative Quantitation of Gene Expression

Real-Time quantitative PCR with fluorescent Taqman probes is a quantitation detection system utilizing the 5'- 3' nuclease activity of Taq DNA polymerase. The method uses an internal fluorescent oligonucleotide probe (Taqman) labeled with a 5' reporter dye and a downstream, 3' quencher dye. During PCR, the 5'-3' nuclease activity of Taq DNA polymerase releases the reporter, whose fluorescence can then be detected

by the laser detector of the Model 7700 Sequence Detection System (PE Applied Biosystems, Foster City, CA, USA).

Amplification of an endogenous control was used to standardize the amount of sample RNA added to the reaction and normalize for Reverse Transcriptase (RT) efficiency. Either cyclophilin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S ribosomal RNA (rRNA) was used as this endogenous control. To calculate relative quantitation between all the samples studied, the target RNA levels for one sample were used as the basis for comparative results (calibrator). Quantitation relative to the "calibrator" can be obtained using the standard curve method or the comparative method (User Bulletin #2: ABI PRISM 7700 Sequence Detection System).

The tissue distribution and the level of the target gene was evaluated for every example in normal and cancer tissue. Total RNA was extracted from normal tissues, cancer tissues, and from cancers and the corresponding matched adjacent tissues. Subsequently, first strand cDNA was prepared with reverse transcriptase and the polymerase chain reaction was done using primers and Taqman probe specific to each target gene. The results are analyzed using the ABI PRISM 7700 Sequence Detector. The absolute numbers are relative levels of expression of the target gene in a particular tissue compared to the calibrator tissue.

Comparative Examples

Similar mRNA expression analysis for genes coding for the diagnostic markers PSA (Prostate Specific Antigen) and PLA2 (Phospholipase A2) was performed for comparison. PSA is the only cancer screening marker available in clinical laboratories. When the panel of normal pooled tissues was analyzed, PSA was expressed at very high levels in prostate, with a very low expression in breast and testis. After more than 55 matching samples from 14 different tissues were analyzed, the data corroborated the tissue specificity seen with normal tissue samples. PSA expression was compared in

cancer and normal adjacent tissue for 12 matching samples of prostate tissue. The relative levels of PSA were higher in 10 cancer samples (83%). Clinical data recently obtained support the utilization of PLA2 as a staging marker for late stages of prostate cancer. mRNA expression data showed overexpression of the mRNA in 8 out of the 12 prostate matching samples analyzed (66%). The tissue specificity for PLA2 was not as good as the one described for PSA. In addition to prostate, also small intestine, liver, and pancreas showed high levels of mRNA expression for PLA2.

Measurement of SEQ ID NO: 1; Clone ID1517021; Gene ID236347 (Cln117)

The absolute numbers shown in Table 2 are relative levels of expression of Cln117 in 12 normal different tissues. All the values are compared to normal testis (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 2: Relative levels of Cln117 Expression in Pooled Samples

Tissue	NORMAL
Colon-Ascending	238
Endometrium	0
Kidney	0.02
Liver	0
Ovary	0.23
Pancreas	0
Prostate	0.06
Small Intestine	35
Spleen	0.0
Stomach	16
Testis	1
Uterus	0.06

The relative levels of expression in Table 2 show that Cln117 mRNA expression is higher (238) in colon compared with all the other normal tissues analyzed. Small intestine, with a relative expression level of 35, and stomach with 16 are the

only other tissues expressing mRNA for Cln117. These results establish that Cln117 mRNA expression is highly specific for tissues from the digestive system.

The absolute numbers in Table 2 were obtained analyzing pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 3.

The absolute numbers depicted in Table 3 are relative levels of expression of Cln117 in 75 pairs of matching samples. All the values are compared to normal testis (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

Table 3: Relative levels of Cln117 Expression in Individual Samples

Sample ID	Cancer Type	Tissue	Cancer	Matching Normal
Sto AC93		Stomach 1	94	189
Sto AC99		Stomach 2	21	30
Sto 539S		Stomach 3	3	4
Sto 728A		Stomach 4	0.11	2
Sto AC44		Stomach 5	20	28
Sto MT54		Stomach 6	58	14
Sto TA73		Stomach 7	88	102
Sto 288S		Stomach 8	44	2
SmI H89		Sm. Int. 1	101	167
SmI 21XA		Sm. Int. 2	62	15
Cln AS45	Adenocarcinoma Duke's Stage A	Colon-Ascending 1	45	57
Cln CM67	Adenocarcinoma Duke's Stage B	Colon-Cecum 2	44	37

Cln AS67	Adenocarcinoma Duke's Stage B	Colon-Ascending 3	97	40
Cln AS43	Adenocarcinoma Duke's Stage C	Colon-Ascending 4	143	39
Cln AS46	Adenocarcinoma Duke's Stage C	Colon Ascending 5	214	182
Cln AS98	Adenocarcinoma Duke's Stage C	Colon-Ascending 6	189	106
Cln B56	Adenocarcinoma Duke's Stage C	Colon-Cecum 7	89	143
Cln AS89	Adenocarcinoma Duke's Stage D	Colon-Ascending 8	45	10
Cln TX01	Adenocarcinoma Duke's Stage B	Colon-Transverse 9	20	42
Cln TX89	Adenocarcinoma Duke's Stage B	Colon-Transverse 10	32	17
Cln TX67	Adenocarcinoma Duke's Stage C	Colon-Transverse 11	23	30
Cln MT38	Adenocarcinoma Duke's Stage D	Colon-Splenic flexture 12	87	82
Cln SG36	Adenocarcinoma Duke's Stage B	Colon-Sigmoid 13	173	144
Cln SG27	Adenocarcinoma Duke's Stage B	Colon-Sigmoid 14	79	76
Cln SG89	Adenocarcinoma Duke's Stage B	Colon-Sigmoid 15	57	56
Cln SG67	Adenocarcinoma Duke's Stage C	Colon-Sigmoid 16	20	19
Cln SG33	Adenocarcinoma Duke's Stage C	Colon-Sigmoid 17	125	223
Cln SG45	Adenocarcinoma Duke's Stage D	Colon-Sigmoid 18	62	48
Cln B34	Adenocarcinoma Duke's Stage A	Colon-Rectosigmoid 19	37	11
Cln CXGA	Adenocarcinoma Duke's Stage A	Colon-Rectum 20	201	136
Cln RC67	Adenocarcinoma Duke's Stage B	Colon-Rectum 21	15	52

Cln SG98	Adenocarcinoma Duke's Stage C	Colon-Rectosigmoid 22	40	58
Cln C9XR	Adenocarcinoma Duke's Stage C	Colon-Rectosigmoid 23	22	27
Cln RS45	Adenocarcinoma Duke's Stage C	Colon-Rectosigmoid 24	269	112
Cln RC01	Adenocarcinoma Duke's Stage C	Colon-Rectum 25	19	62
Cln RC89	Adenocarcinoma Duke's Stage D	Colon-Rectum 26	0.36	44
Cln RC24	Adenocarcinoma Duke's Stage D	Colon-Rectum 27	91	77
Bld 32XK		Bladder 1	0.82	0
Bld 46XK		Bladder 2	0	0.25
Bld 66X		Bladder 3	0.35	0
Cvx NKS54		Cervix 1	0.31	0
Cvx KS52		Cervix 2	0	0
Cvx NK24		Cervix 3	0.23	0
End 4XA		Endometrium 1	0	0
End 8911		Endometrium 2	0.04	1.24
End 8XA		Endometrium 3	0.08	4
Kid 5XD		Kidney 1	0.92	1.67
Kid 6XD		Kidney 2	0.30	0.02
Kid 106XD		Kidney 3	0	0.06
Kid 126XD		Kidney 4	0	0
Kid 12XD		Kidney 5	0	0
Liv 42X		Liver 1	50	0
Liv 15XA		Liver 2	16	0.19
Liv 94XA		Liver 3	0.37	0.04

Lng AC69		Lung 1	0.41	0
Lng BR94		Lung 2	0.05	0
Lng 47XQ		Lung 3	0	0
Lng 90X		Lung 4	0	0
Mam 59X		Mammary Gland 1	0	0
Mam 12X		Mammary Gland 2	0	0
Mam B011X		Mammary Gland 3	0	0
Mam A06X		Mammary Gland 4	0.02	0
Ovr 103X		Ovary 1	0.01	0.021
Pan 71XL		Pancreas 1	114.56	123
Pan 77X		Pancreas 2	0.18	0.09
Pan 92X		Pancreas 3	146	0.30
Pan 82XP		Pancreas 4	0.02	0
Pro 109XB		Prostate 1	0	0.01
Pro 34B		Prostate 2	0	0.03
Pro 12B		Prostate 3	0	0
Pro 23B		Prostate 4	0	0.05
Tst 39X		Testis 1	1.60	0.60
Utr 85XU		Uterus 1	0.21	0
Utr 141XO		Uterus 2	1.80	0
Utr 23XU		Uterus 3	1.36	0.07

0=negative

In the analysis of matching samples, the higher levels of expression were in colon, showing a high degree of tissue specificity for digestive system. Of all the samples different than colon analyzed, only four samples (the cancer samples for the matches of liver 1 and 2, and pancreas 1 and 3; and the normal adjacent for the pancreas match #1) showed

an expression comparable to the mRNA expression in colon. These results confirm the tissue specificity results obtained with the panel of normal pooled samples (Table 2).

Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 3 shows overexpression of Cln117 in 17 primary colon cancer tissues compared with their respective normal adjacent (colon samples #2, 3, 4, 5, 6, 8, 10, 12, 13, 14, 15, 16, 18, 19, 20, 24, and 27). There was overexpression in the cancer tissue for 63% of the colon matching samples tested (total of 27 colon matching samples).

Altogether, the high level of tissue specificity, plus the mRNA overexpression in 63% of the primary colon matching samples tested is demonstrative of Cln117 being a diagnostic marker for colon cancer.

Measurement of SEQ ID NO:2; Clone ID776410; Gene ID202109 (Cln124)

The absolute numbers depicted in Table 4 are relative levels of expression of Cln124 in 12 normal different tissues. All the values are compared to normal colon (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 4: Relative levels of Cln124 Expression in Pooled Samples

Tissue	NORMAL
Colon-Ascending	10000
Endometrium	0
Kidney	0.2
Liver	0
Ovary	0
Pancreas	0
Prostate	0.3
Small Intestine	6

Spleen	2
Stomach	0
Testis	1
Uterus	0

The relative levels of expression in Table 4 show that Cln124 mRNA expression is more than 1000 fold higher in the pool of normal colon (10000) compared to all the other tissues analyzed. These results demonstrate that Cln124 mRNA expression is highly specific for colon.

The absolute numbers in Table 4 were obtained analyzing pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 5.

The absolute numbers depicted in Table 5 are relative levels of expression of Cln124 in 41 pairs of matching samples. All the values are compared to normal colon (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

Table 5: Relative levels of Cln124 Expression in Individual Samples

Sample ID	Cancer Type	Tissue	Cancer	Matching Normal
Sto MT54		Stomach 1	0	0
SmI 21XA		Sm. Int. 1	0	0
Cln AS45	Adenocarcinoma Duke's Stage A	Colon-Ascending 1	0.03	0.15
Cln CM67	Adenocarcinoma Duke's Stage B	Colon-Cecum 2	0.37	2.06
Cln AS12	Adenocarcinoma Duke's Stage B	Colon-Ascending 3	0.40	5.20
Cln AS43	Adenocarcinoma Duke's Stage C	Colon-Ascending 4	0	0.10

Cln AS46	Adenocarcinoma Duke's Stage C	Colon Ascending 5	0.02	1.73
Cln AS98	Adenocarcinoma Duke's Stage C	Colon-Ascending 6	0.17	1.58
Cln AC19	Adenocarcinoma Duke's Stage D	Colon-Ascending 7	0.59	7.05
Cln TX01	Adenocarcinoma Duke's Stage B	Colon-Transverse 8	0	1.53
Cln MT38	Adenocarcinoma Duke's Stage D	Colon-Splenic flexture 9	0.001	2.43
Cln DC19	Adenocarcinoma Duke's Stage B	Colon-Descending 10	0.41	1.34
Cln DC63	Adenocarcinoma Duke's Stage C	Colon-Descending 11	0.005	0.50
Cln DC22	Adenocarcinoma Duke's Stage D	Colon-Descending 12	0.002	0.09
Cln SG36	Adenocarcinoma Duke's Stage B	Colon-Sigmoid 13	0.03	0.81
Cln SG20	Adenocarcinoma Duke's Stage B	Colon-Sigmoid 14	0	1.64
Cln SG27	Adenocarcinoma Duke's Stage B	Colon-Sigmoid 15	0.11	1.04
Cln SG89	Adenocarcinoma Duke's Stage B	Colon-Sigmoid 16	0.11	1.07
Cln SG66	Adenocarcinoma Duke's Stage C	Colon-Sigmoid 17	0	0.45
Cln SG67	Adenocarcinoma Duke's Stage C	Colon-Sigmoid 18	0.02	0.04
Cln SG33	Adenocarcinoma Duke's Stage C	Colon-Sigmoid 19	0.03	1.00
Cln CXGA	Adenocarcinoma Duke's Stage A	Colon-Rectum 20	0.34	2.36
Cln RC24	Adenocarcinoma Duke's Stage B	Colon-Rectum 21	0.86	1.64
Cln RS86	Adenocarcinoma Duke's Stage B	Colon-Rectosigmoid 22	0.01	0.97

Cln RS16	Adenocarcinoma Duke's Stage B	Colon-Rectosigmoid 23	0.01	0.05
Cln SG98	Adenocarcinoma Duke's Stage C	Colon-Rectosigmoid 24	0.43	2.77
Cln C9XR	Adenocarcinoma Duke's Stage C	Colon-Rectosigmoid 25	0.01	0.35
Cln RS53	Adenocarcinoma Duke's Stage C	Colon-Rectosigmoid 26	0.01	1.60
Cln RS45	Adenocarcinoma Duke's Stage C	Colon-Rectosigmoid 27	0.23	0.54
Cln RC24	Adenocarcinoma Duke's Stage D	Colon-Rectum 28	0.86	1.64
Bld 32XK		Bladder 1	0	0
Cvx KS52		Cervix 1	0	0
End 10479		Endometrium 1	0	0
Kid 109XD		Kidney 1	0	0
Kid 107XD		Kidney 2	0	0
Kid 106XD		Kidney 3	0	0
Liv 15XA		Liver 1	0	0
Lng 47XQ		Lung 1	0	0
Mam 12X		Mammary Gland 1	0	0
Tst 39X		Testis 1	0	0
Utr 85XU		Uterus 1	0	0

0=negative

In the analysis of matching samples, the higher levels of expression were in colon showing a high degree of tissue specificity for colon tissue. These results confirm the

tissue specificity results obtained with normal pooled samples (Table 4).

Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. lower levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 5 shows reduction of expression of Cln124 in 28 primary colon cancer samples compared with their respective normal adjacent. There is downregulation of Cln124 in the cancer tissue for all the colon matching samples tested (total of 28 primary colon matching samples).

Altogether, the high level of tissue specificity, plus the mRNA downregulation in 100% of the colon matching samples tested are demonstrative of Cln124 being a diagnostic marker for colon cancer.

Measurement of SEQ ID NO:3; Clone ID611082; Gene ID202298 (Cln125)

The absolute numbers depicted in Table 6 are relative levels of expression of Cln125 in 12 normal different tissues. All the values are compared to normal testis (calibrator). These RNA samples are commercially available pools, originated by pooling samples of a particular tissue from different individuals.

Table 6: Relative levels of Cln125 Expression in Pooled Samples

Tissue	NORMAL
Colon-Ascending	2486
Endometrium	0.8
Kidney	0
Liver	0
Ovary	1.3
Pancreas	0
Prostate	0
Small Intestine	0
Spleen	0
Stomach	0.5

Testis	1
Uterus	0

The relative levels of expression in Table 6 show that Cln125 mRNA expression is higher (2486) in colon compared with all the other normal tissues analyzed. Ovary, with a relative expression level of 1.3, endometrium (0.8), and stomach (0.5) are the only other tissues expressing mRNA for Cln125. These results established that Cln125 mRNA expression is highly specific for colon.

The absolute numbers in Table 6 were obtained analyzing pools of samples of a particular tissue from different individuals. They can not be compared to the absolute numbers originated from RNA obtained from tissue samples of a single individual in Table 7.

The absolute numbers depicted in Table 7 are relative levels of expression of Cln125 in 75 pairs of matching samples. All the values are compared to normal testis (calibrator). A matching pair is formed by mRNA from the cancer sample for a particular tissue and mRNA from the normal adjacent sample for that same tissue from the same individual.

Table 7: Relative levels of Cln125 Expression in Individual Samples

Sample ID	Cancer Type	Tissue	Cancer	Matching Normal
Sto AC93		Stomach 1	1	2
Sto AC99		Stomach 2	0	0
Sto 539S		Stomach 3	0	0
Sto 728A		Stomach 4	0	0
Sto AC44		Stomach 5	0	0
Sto MT54		Stomach 6	3	0
Sto TA73		Stomach 7	0	0
Sto 288S		Stomach 8	0	0
SmI H89		Sm. Int. 1	0	0.5

SmI 21XA		Sm. Int. 2	1	0
Cln CM67	Adenocarcinoma Duke's Stage B	Colon-Cecum 1	3	27
Cln AS12	Adenocarcinoma Duke's Stage B	Colon-Ascending 2	106	1290
Cln AS43	Adenocarcinoma Duke's Stage C	Colon-Ascending 3	0	131
Cln AS46	Adenocarcinoma Duke's Stage C	Colon Ascending 4	0	461
Cln AS98	Adenocarcinoma Duke's Stage C	Colon-Ascending 5	376	558
Cln B56	Adenocarcinoma Duke's Stage C	Colon-Cecum 6	32	572
Cln AS89	Adenocarcinoma Duke's Stage D	Colon-Ascending 7	3	0
Cln AC19	Adenocarcinoma Duke's Stage D	Colon-Ascending 8	2	603
Cln TX01	Adenocarcinoma Duke's Stage B	Colon-Transverse 9	1	525
Cln TX89	Adenocarcinoma Duke's Stage B	Colon-Transverse 10	5	401
Cln TX67	Adenocarcinoma Duke's Stage C	Colon-Transverse 11	0	717
Cln MT38	Adenocarcinoma Duke's Stage D	Colon-Splenic flexture 12	3	1562
Cln SG36	Adenocarcinoma Duke's Stage B	Colon-Sigmoid 13	3	1073
Cln SG20	Adenocarcinoma Duke's Stage B	Colon-Sigmoid 14	2	1021
Cln SG27	Adenocarcinoma Duke's Stage B	Colon-Sigmoid 15	207	951
Cln SG89	Adenocarcinoma Duke's Stage B	Colon-Sigmoid 16	14	263
Cln SG67	Adenocarcinoma Duke's Stage C	Colon-Sigmoid 17	18	32
Cln SG33	Adenocarcinoma Duke's Stages C	Colon-Sigmoid 18	43	1075

	Cln B34	Adenocarcinoma Duke's Stage A	Colon-Rectosigmoid 19	1	56
	Cln CXGA	Adenocarcinoma Duke's Stage A	Colon-Rectum 20	95	1041
	Cln RC67	Adenocarcinoma Duke's Stage B	Colon-Rectum 21	32	207
	Cln SG98	Adenocarcinoma Duke's Stage C	Colon-Rectosigmoid 22	223	2781
5	Cln C9XR	Adenocarcinoma Duke's Stage C	Colon-Rectosigmoid 23	1	277
	Cln RS45	Adenocarcinoma Duke's Stage C	Colon-Rectosigmoid 24	535	513
	Cln RC89	Adenocarcinoma Duke's Stage D	Colon-Rectum 25	0	157
	Cln RC24	Adenocarcinoma Duke's Stage D	Colon-Rectum 26	232	346
	Bld 32XK		Bladder 1	2	0
10	Bld 46XK		Bladder 2	0	0
	Bld 66X		Bladder 3	0	0
	Cvx NKS54		Cervix 1	0	0
	Cvx KS52		Cervix 2	0	0
15	Cvx NK24		Cervix 3	0	0
	End 10479		Endometrium 1	0	0
	End 8911		Endometrium 2	0	0
	End 8XA		Endometrium 3	0	0
20	Kid 5XD		Kidney 1	0	0
	Kid 109XD		Kidney 2	0	0
	Kid 107XD		Kidney 3	0	0
25	Kid 6XD		Kidney 4	0	0

Kid 106XD		Kidney 5	0	0
Kid 126XD		Kidney 6	0	0
Kid 12XD		Kidney 7	0	0
Liv 42X		Liver 1	0	0
Liv 15XA		Liver 2	0	0
Liv 94XA		Liver 3	0	0
Lng AC69		Lung 1	0	0
Lng BR94		Lung 2	0	0
Lng 47XQ		Lung 3	0	0
Lng 90X		Lung 4	0	0
Mam 59X		Mammary Gland 1	0	0
Mam 12X		Mammary Gland 2	0	0
Mam B011X		Mammary Gland 3	0	0
Mam A06X		Mammary Gland 4	0	0
Pan 71XL		Pancreas 1	1	0.12
Pan 77X		Pancreas 2	0	0
Pan 92X		Pancreas 3	0	0
Pan 82XP		Pancreas 4	0	0
Pro 109XB		Prostate 1	0	0
Pro 34B		Prostate 2	0.48	0.23
Pro 12B		Prostate 3	0.36	0
Pro 23B		Prostate 4	0.33	0
Tst 39X		Testis 1	0	7.67
Utr 85XU		Uterus 1	0	0
Utr 141XO		Uterus 2	0	0
Utr 23XU		Uterus 3	0	0

0=negative

In the analysis of matching samples, the higher levels of expression were in colon, showing a high degree of tissue specificity for colon tissue. Of all the samples different than colon analyzed, only one sample (the cancer sample Liver 2 with 48.6) showed an expression comparable to the mRNA expression in colon. These results confirm the tissue specificity results obtained with the panel of normal pooled samples (Table 6).

Furthermore, the level of mRNA expression in cancer samples and the isogenic normal adjacent tissue from the same individual were compared. This comparison provides an indication of specificity for the cancer stage (e.g. higher or lower levels of mRNA expression in the cancer sample compared to the normal adjacent). Table 7 shows the reduction of mRNA levels of Cln125 in 24 primary colon cancer tissues compared with their respective normal adjacent (colon samples #1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, and 26). There was overexpression in the cancer tissue for two of the colon matching samples tested (total of 26 colon matching samples).

Altogether, the high level of tissue specificity, plus the dramatic reduction of mRNA levels of Cln125 in the majority (92%) of the colon cancer samples in the matching pairs tested are demonstrative of Cln125 being a diagnostic marker for colon cancer.

What is claimed is:

1. A method for diagnosing the presence of colon cancer in a patient comprising:

(a) measuring levels of CSG in cells, tissues or bodily fluids in said patient; and

(b) comparing the measured levels of CSG with levels of CSG in cells, tissues or bodily fluids from a normal human control, wherein an increase in measured levels of CSG in said patient versus normal human control is associated with the presence of colon cancer.

2. A method of diagnosing metastatic colon cancer in a patient comprising:

(a) identifying a patient having colon cancer that is not known to have metastasized;

(b) measuring CSG levels in a sample of cells, tissues, or bodily fluid from said patient for CSG; and

(c) comparing the measured CSG levels with levels of CSG in cell, tissue, or bodily fluid type of a normal human control, wherein an increase in measured CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

3. A method of staging colon cancer in a patient having colon cancer comprising:

(a) identifying a patient having colon cancer;

(b) measuring CSG levels in a sample of cells, tissues, or bodily fluid from said patient; and

(c) comparing measured CSG levels with levels of CSG in cells, tissues, or bodily fluid type of a normal human control sample, wherein an increase in measured CSG levels in said patient versus the normal human control is associated with a cancer which is progressing and a decrease in the measured CSG levels is associated with a cancer which is regressing or in remission.

4. A method of monitoring colon cancer in a patient for the onset of metastasis comprising:

(a) identifying a patient having colon cancer that is not known to have metastasized;

(b) periodically measuring levels of CSG in samples of cells, tissues, or bodily fluid from said patient for CSG; and

(c) comparing the periodically measured CSG levels with levels of CSG in cells, tissues, or bodily fluid type of a normal human control, wherein an increase in any one of the periodically measured CSG levels in the patient versus the normal human control is associated with a cancer which has metastasized.

5. A method of monitoring the change in stage of colon cancer in a patient comprising:

(a) identifying a patient having colon cancer;

(b) periodically measuring levels of CSG in cells, tissues, or bodily fluid from said patient for CSG; and

(c) comparing the periodically measured CSG levels with levels of CSG in cells, tissues, or bodily fluid type of a normal human control, wherein an increase in any one of the periodically measured CSG levels in the patient versus the normal human control is associated with a cancer which is progressing in stage and a decrease is associated with a cancer which is regressing in stage or in remission.

6. The method of claim 1, 2, 3, 4 or 5 wherein the CSG comprises SEQ ID NO:1, 2 or 3.

7. An antibody against an CSG wherein said CSG comprises SEQ ID NO:1, 2 or 3.

8. A method of imaging colon cancer in a patient comprising administering to the patient an antibody of claim 7.

9. The method of claim 8 wherein said antibody is labeled with paramagnetic ions or a radioisotope.

10. A method of treating colon cancer in a patient comprising administering to the patient an antibody of claim 7.

11. The method of claim 10 wherein the antibody is conjugated to a cytotoxic agent.

SEQUENCE LISTING

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<120> A NOVEL METHOD OF DIAGNOSING, MONITORING, STAGING, IMAGING AND TREATING COLON CANCER

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Docket No.
DEX-0150

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

A Novel Method of Diagnosing, Monitoring, Staging, Imaging and Treating Colon Cancer

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on **20 July 1999** as United States Application No. or PCT International Application Number **PCT/US99/16357**

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

60/095,231	4 August 1998
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

3-00

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Sixth inventor's signature Date
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